Green synthesis of new neurotransmitters based on isatin moiety

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE
IN
BIOTECHNOLOGY

Submitted By
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Date: 20- May-2025

To whome it may concern

This is to certify that Miss. Pradipta Malik, pursuing a M.Sc. in Biotechnology, Sister Nivedita University, Kolkata, has successfully completed her internship under my guidance from January, 2025 to May, 2025 at Institute of Health Sciences, Presidency University, Kolkata, on ''Green synthesis of new neurotransmitters based on isatin moiety''.

Pradipta is very sincere, punctual, disciplined and obedient. I have always noticed a great deal of determination in Pradipta and she is an enthusiastic student who always strives for new knowledge. I wish her every success in life.

Sincerely,

Piyali Sarkar

Dr. Piyali Sarkar

Dr. Piyali Sarkar
Assistant Professor
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Supervisor's approval

This is to certify that the thesis titled '*Green synthesis of new neurotransmitters based on isatin moiety*' submitted by Pradipta Malik from Sister Nivedita University, Kolkata, has been reviewed and approved for submission in partial fulfilment of the requirements for the degree Master of Science in Biotechnology.

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DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "Green synthesis of new neurotransmitters based on isatin moiety" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Biotechnology is an authentic record of my own work carried out during the period from 13 January 2025 to 20 January 2025 under the supervision of Dr. Piyali Sarkar Department of Biotechnology, Institute of Health Sciences.

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Pradipta Malik

06.06.2025

(Signature of the students with Date)

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TO WHOM IT MAY CONCERN

This is to certify that the project report titled "Green synthesis of new neurotransmitters based on isatin moiety", submitted by Ms. Pradipta Malik (Registration No. 230020417883 Enrolment No. 2322210002041) to the Department of Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by her for the partial fulfilment of the requirements for the Master of Science degree.

Date: 03.06.2025

Place: SISTER NIVEDITA UNIVERSITY

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Acknowledgement

First and foremost, I would like to express my sincere gratitude to my supervisor Dr. Piyali Sarkar, for her valuable guidance, constant support, and immense knowledge throughout my M.Sc. internship. Her insightful advice and encouragement have been crucial in the successful completion of this research.

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Abbreviations

Abbreviation	*Meaning*
BBB	Blood-Brain barrier
CNS	Central Nervous System
NTs	Neurotransmitters
NMR	Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
ppm	Parts per million
UV	Ultraviolet
Cu	Copper
DMSO	Dimethyl sulfoxide
No.	Number
&	And
FBS	Fetal Bovine Serum
DMF	Dimethyl formamide
DMEM	Dulbecco's Modified Eagle Medium
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
RB	Round bottom flask

Abstract

Neurotransmitters are the body's chemical messengers, transmitting signals across synapses from one neuron to another, profoundly influencing everything from mood and sleep to learning and pain perception. Among these, Serotonin (5-hydroxytryptamine or 5-HT) stands out as a critically important monoamine neurotransmitter. It orchestrates a vast array of physiological, cognitive, and immune functions, with its dysregulation intricately linked to numerous severe psychiatric disorders like depression and anxiety, . However, a formidable barrier in developing effective therapeutics for these Central Nervous System (CNS) related disorders is the blood-brain barrier (BBB). This highly selective semipermeable membrane protects the brain from circulating toxins and pathogens, but simultaneously restricts the passage of many therapeutic agents, including exogenous serotonin, from reaching their targets within the brain. This limitation severely hampers the direct pharmacological manipulation of central serotonin levels, impacting treatment efficacy.

With an eye towards designing pharmacophores capable of mimicking serotonin and efficiently traversing the BBB, this thesis investigates the green synthesis of new neurotransmitter analogues based on the isatin moiety. Our approach meticulously prioritizes environmentally friendly methodologies, embodying the principles of green chemistry for sustainable drug discovery. We propose a synthetic approach to construct new chemical entities where the isatin core acts as a versatile scaffold. This abstract explains the reasoning behind integrating the isatin moiety, known for its diverse biological activities and structural versatility, into novel compounds aimed at imitating serotonin's pharmacological profile. Furthermore, this thesis aims to design molecules in innovative ways, fulfilling the ultimate goal of developing a new generation of CNS-active compounds with improved efficacy and reduced side effects, for promoting advanced therapeutic interventions in neurological and psychiatric conditions. This work not only contributes to medicinal chemistry but also champions eco-conscious pharmaceutical development.

KEYWORDS – Neurotransmitters, Green synthesis, Serotonin, Blood-brain barrier, isatin, TLC, NMR

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1. INTRODUCTION

The brain's extraordinary range of functions is facilitated by the intercellular communication of neurons, largely accomplished via chemical synaptic transmission [1]. This is done by neurotransmitters which are the body's chemical messengers. Neurotransmitters play a crucial role in the functioning of the nervous system which oversees the control and coordination of all bodily activities [2]. A traveling action potential at the axon terminal, induces the synaptic release of neurotransmitters [2]. Eventually the neurotransmitter diffuses across the synapse and binds to receptors on the postsynaptic neuron. This interaction can either excite or inhibit the activity of the postsynaptic neuron [2].

Importance of neurotransmitters

Neurotransmitters are fundamental for control of behavioural and physiological functions in the body that are essential for survival [3]. The functions of neurotransmitters are directly linked to the central nervous system that controls the human body and directly supervise neurophysiological processes such as sleep, emotions, memory, and other cognitive functions [4,5]. They play essential roles in the functioning of the brain, being fundamental regulators of neuronal growth, differentiation, and survival. Consequently, abnormal levels of NTs are reflected in dysregulation of brain functions, leading to various physical, psychotic, and neurodegenerative diseases [6]. Some CNS-related disorders such as epilepsy, multiple sclerosis, Alzheimer's and Parkinson's disease can occur due to NT imbalances.

Serotonin, or 5-hydroxytryptamine is one such important monoamine neurotransmitter which plays a crucial role in various physiological functions such as regulating mood, sleep patterns, sexuality, anxiety, appetite and pain. Serotonin is a key messenger in the brain that is associated with behaviour and cognitive functions [7]. Serotonin, in conjunction to other neurotransmitters, like dopamine, affects sleep quality and also modulates glutamate and GABA transmission [8]. Serotonin affects a wide variety of immune functions; it can affect systemic inflammation, as well as a number of autoimmune diseases [9]. Serotonin regulates behavior by modulating neuronal excitability and plasticity, and its dysfunction has been implicated in several psychiatric disorders, such as impulsive aggression, anxiety and depression [10]. Serotonin modulation can, therefore, be potentially useful as a therapeutic strategy [9].

Problems associated with neurotransmitters

There are many problems associated with neurotransmitters. A significant challenge is serotonin's inability to cross the blood-brain barrier [9].

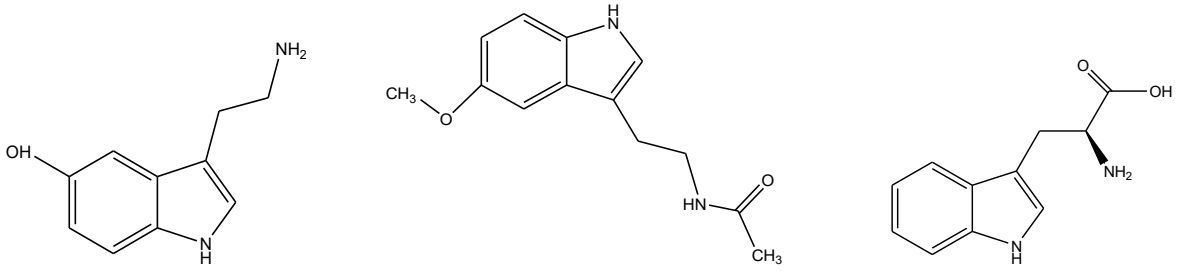
The blood–brain barrier (BBB), made up of endothelial cells, is a dynamic boundary between the brain and the surrounding tissues which plays a critical role in maintaining normal physiological functions of the brain. Endothelial cells strictly regulate the movement of substances into and out of the brain through tight junctions, maintaining the selective permeability of the blood–brain barrier [11]. It safeguards the CNS against circulating toxins, pathogens, and xenobiotics [12]. Additionally, it supplies the brain with nutrients and expels waste products back into the bloodstream [12]. The BBB establishes a very delicate ion homeostasis and adjusts the level of neurotransmitters, which guarantee ideal function of the CNS [12]. It also prevents many therapeutic agents from reaching their targets in the brain that have been developed and could be used to treat various neurological disorders [12,13]. The blood–brain barrier greatly restricts influx of some neurotransmitters such as serotonin but allows the influx of many others [14].

This research focuses on creating new Pharmacophore designed to replicate the function of serotonin, with the aim of treating neurodegenerative disorders like depression, Parkinson's disease, and Alzheimer's disease. It aims to develop a Pharmacophore that could more effectively mimic serotonin's effects and be easily capable to cross the BBB. The primary compounds utilized in this reaction was isatin and amines as starting materials.

2. AIMS AND OBJECTIVE

1) *Design and Synthesis of the compound*: One important aspect of my research involves designing and the synthesis of the compound using green chemistry, focusing on environmental stability.
    - This process reduces chemical waste and minimizes the need for extensive purification steps.
    - Copper was used as catalyst, which was recovered and reused, promoting resource conservation.
2) *Characterization of the compound*:
    - Nuclear Magnetic Resonance (NMR) spectroscopy was used to determine the molecular structure of the synthesized compound.
    - The new pharmacophores were fully purified and characterized before proceeding to biological tests.
3) *Biological evaluation of the compound:*
    - The synthesized compound represents a probable advanced therapeutic agent.
    - Ongoing biological testing aims to evaluate the pharmacophores' therapeutic potential, particularly their ability to mimic serotonin in natural cells.

3. DESIGNING ASPECT



Serotonin **Melatonin** **Tryptophan**

Fig 1: Amine based neurotransmitters

The 3 neurotransmitters shown in Fig 1, Tryptophan is the precursor of serotonin and melatonin. All of them share some common functional groups which are the indole ring and amine (-NH2). But among these 3, only serotonin cannot cross the blood brain barrier while melatonin and tryptophan can. So we try to design a model where we can introduce indole ring and amine group.

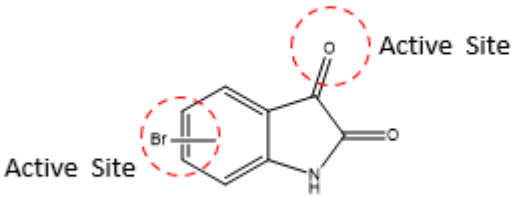


Figure 2: Bromo substituted Isatin ring

We have chosen bromo-substituted isatin moiety which has one indole ring and two active centers for amine incorporation. One is the bromide center, and another one is ketone center. So, we planned to react this molecule with amines such as 2 Picolylamine and N,N-Dimethylaniline. The interaction of these functional groups is expected to form a stable product that further modifies the original indole structure, enhancing the potential to cross the blood-brain barrier.

4. MATERIALS AND METHOD

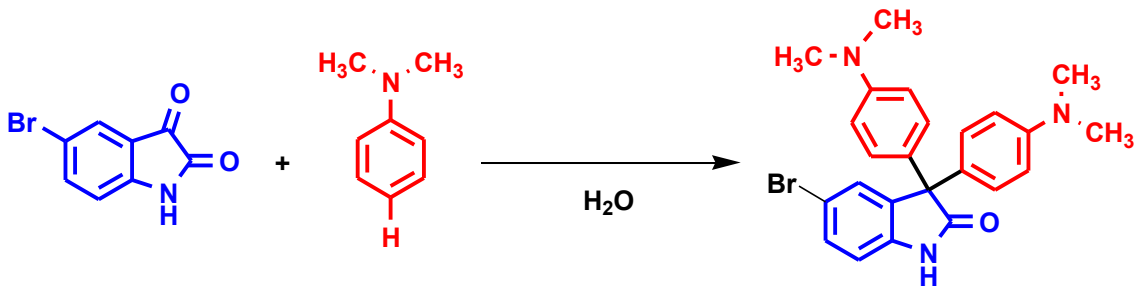
4.1. Materials

5-bromo isatin, N, N-Dimethylaniline, 7-bromo isatin, 2 picolylamine, Hexane, Ethyl acetate, Methanol, Chloroform, DMF, Cu powder, distilled water, TLC plate, silica gel.

Apparatus

Condenser, magnetic stirrer, oil bath, round bottom flasks, conical flasks, Rotary evaporator.

4.2. Reaction 1



5-bromo-3,3-bis(4-(dimethylamino)phenyl)indolin-2-one

5-bromoisatin **N, N-Dimethylaniline**

Figure 3: Reaction 1

4.2.1. Procedure

339 mg 5-bromoisatin, 363mg N, N-Dimethylaniline, approx. 5 ml distilled water and catalytic amount of copper powder were mixed in a 25 ml round bottom flask. Next the flask was fitted in a reflux condenser to prevent solvent loss and maintain a controlled reaction environment. The reaction was carried out in a magnetic stirrer with an oil bath for 18 hours and temperature is gradually raised and maintained at approx.120°C. In this reaction, copper served as a catalyst to facilitate the reaction between 5-Bromoisatin and N, N-Dimethylaniline, with water acting as the solvent. Using water as a solvent fulfils green chemistry principles, as it reduces dependence on harmful organic solvents that can cause harm to environment.

4.2.2. Reaction process

The progress of the reaction was monitored at some intervals by TLC (thin layer chromatography) method. In this process, a small sample of the reaction mixture was taken and spotted onto a TLC plate alongside the starting material and product. The Plate is then developed in an appropriate solvent system. The components on the plate move upwards by capillary action. The new spots are then observed under UV light. The disappearance of the starting material's spot and appearance of new spot corresponding to the desired product indicate the progress of the reaction.

4.2.3. Work up

In this process, initially the water was evaporated using rotary evaporator to get the crude product. The catalyst was then recovered by isolation of crude product using organic solvents such as mixture of ethyl acetate, methanol and chloroform. The organic layer is decanted off for preparation of slurry for column chromatography. The Cu powder remains intact in this mixture.

4.2.4. Purification

Column chromatography technique was used to isolate the nanodrug and eliminate impurities. This technique separates compounds based on their separation of stationary phase (silica) and mobile phase (ethyl acetate and hexane). This approach was particularly effective in distinguishing the desired product from any unreacted starting material or byproducts. TLC was utilized throughout the process to track the reaction's progress and desired compound was being produced.

For the purification of the compound, a clean chromatography column was prepared by washing with pure hexane and then drying. A small plug of cotton was then positioned at the bottom of the column to support the stationary phase. A slurry of silica gel, prepared in hexane, was carefully loaded into the column. Then the compound was loaded in the packed column. Another cotton plug was placed on top of the silica bed leaving some gap between the silica bed and cotton. The column was then eluted with a gradient of hexane and ethyl acetate. The column was eluted with a gradient of hexane and ethyl acetate. The stopcock was opened for the flow of the mobile phase. The eluates are collected in conical flask, poured in an RB and evaporated leaving behind the product. The solvent polarity was gradually increased based on thin-layer chromatography (TLC) results to achieve optimal separation. The purified nanodrug was subsequently collected and prepared for further characterization.

4.3. Reaction 2

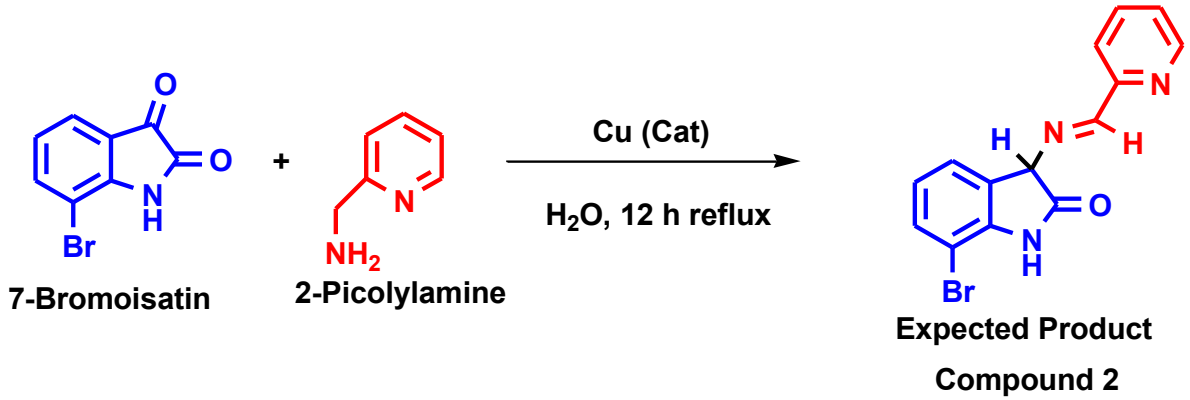


Figure 4: Reaction 2

4.3.1. Procedure

452.06 mg 7-bromo isatin, 420 uL 2 picolylamine, approx. 5 ml distilled water and catalytic amount of copper powder were mixed in a 25 ml round bottom flask. Next the flask was fitted in a reflux condenser to prevent solvent loss and maintain a controlled reaction environment. The reaction was carried out in a magnetic stirrer with an oil bath for 18 hours and temperature is gradually raised and maintained at approx..120°C. In this reaction, copper served as a catalyst to facilitate the reaction between 7-Bromoisatin and 2 picolylamine, with water acting as the solvent. Using water as a solvent fulfils green chemistry principles, as it reduces dependence on harmful organic solvents that can cause harm to environment.

4.3.2. Reaction process

The progress of the reaction was monitored at some intervals by TLC (thin layer chromatography) method. In this process, a small sample of the reaction mixture was taken and spotted onto a TLC plate alongside the starting material and product. The Plate was then developed in an appropriate solvent system made of hexane and ethyl acetate. The components on the plate move upwards by capillary action. The new spots/bands which were formed on the TLC plate, were then observed under the UV light. The disappearance of the starting material's spot and appearance of new spot corresponding to the desired product indicate the progress of the reaction.

4.3.3. Work up

In this process, initially the water was evaporated using rotary evaporator to get the crude product. The catalyst was then recovered by isolation of crude product using organic solvents such as mixture of ethyl acetate, methanol and chloroform. The organic layer is decanted off for preparation of slurry for column chromatography. The Cu powder remains intact in this mixture.

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5. RESULTS AND DISCUSSION

A small sample of the dried product was set aside to be analysed by using NMR spectroscopy. For this, the sample is dissolved in an appropriate solvent (such as DMSO-d_6), transferred it to a clean NMR tube, and prepared according to standard guidelines to ensure accurate and interpretable spectra.

5.1. Characterization of compound 1 using ^{1}H NMR (600 MHz)

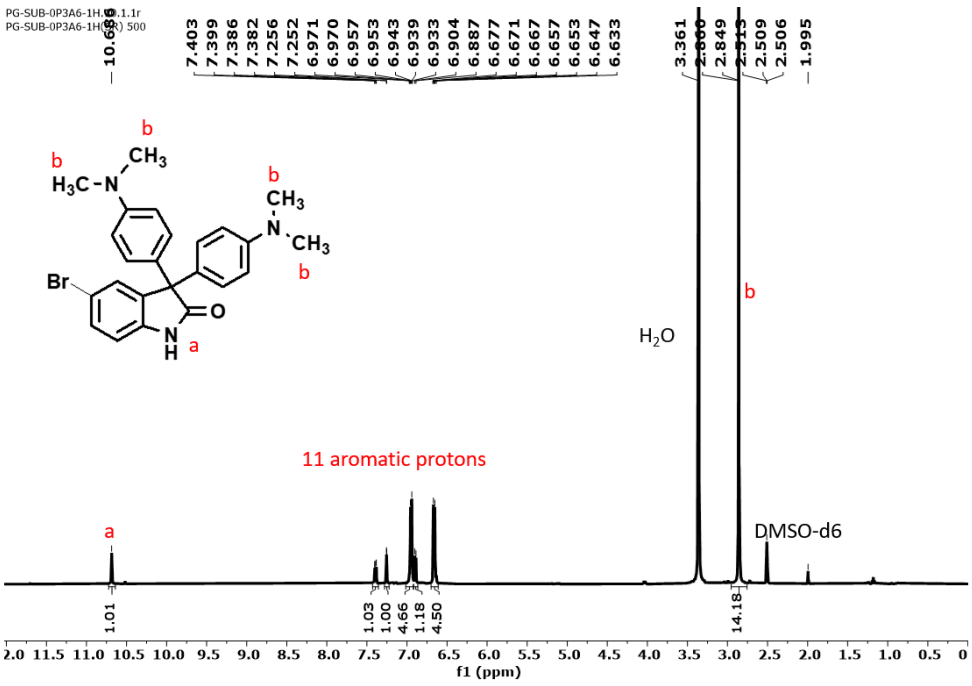


Figure 5: ^{1}H NMR graph of compound 1

The graph above, indicates that –

- On the graph, b at **δ 2.91 ppm** represents the aliphatic protons that is the H from N-CH_3 groups. Total 6H from each group.
- The peaks present between **δ 6.63** and **δ 7.40 ppm** represents the 11 aromatic protons that is the H present in the aromatic rings.
- The peak a at **δ 10.68 ppm** represents an acidic proton, 1H associated with -NH.

5.2. Characterization of compound 1 using ^{13}C NMR (150 MHz)

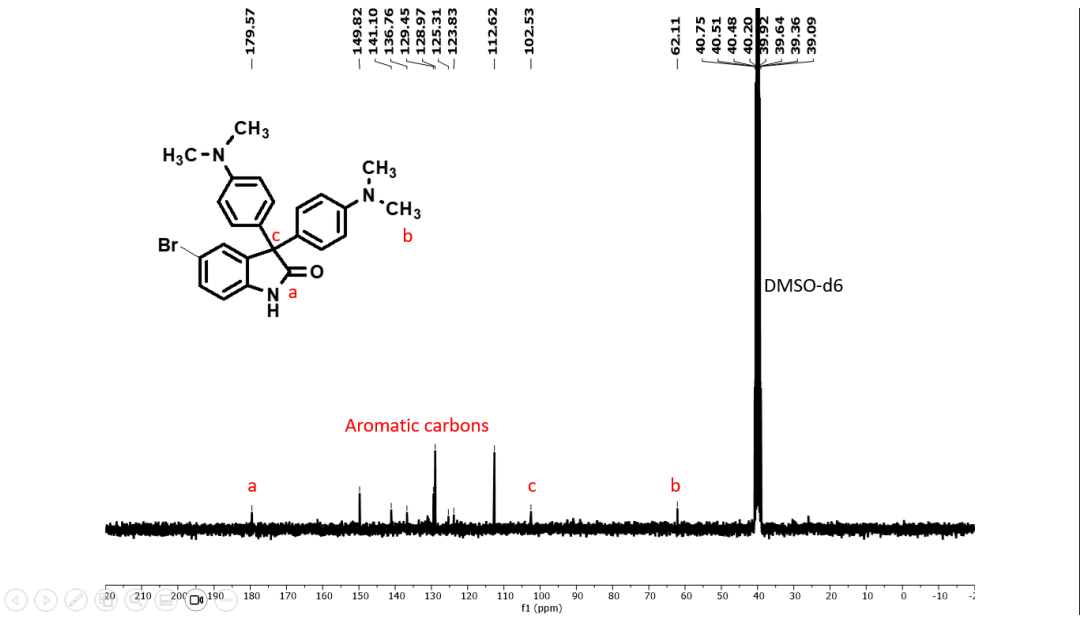


Figure 6: ^{13}C NMR graph of compound 1

The graph above, indicates that –

- On the graph, b at **δ 62.11 ppm** corresponds to a methyl group attached to a Nitrogen atom (N-CH_3).
- The peak c at **δ 102.53 ppm** indicates a quarternary carbon.
- The peaks marked as 'Aromatic carbons' between **δ 112.62 ppm** and **δ 149.82 ppm** represents the Carbon atoms in the aromatic rings.
- The peak a at **δ 179.57 ppm** represents a carbonyl carbon (C=O).

5.3. Characterization of compound 2 using ^{1}H NMR (600 MHz)

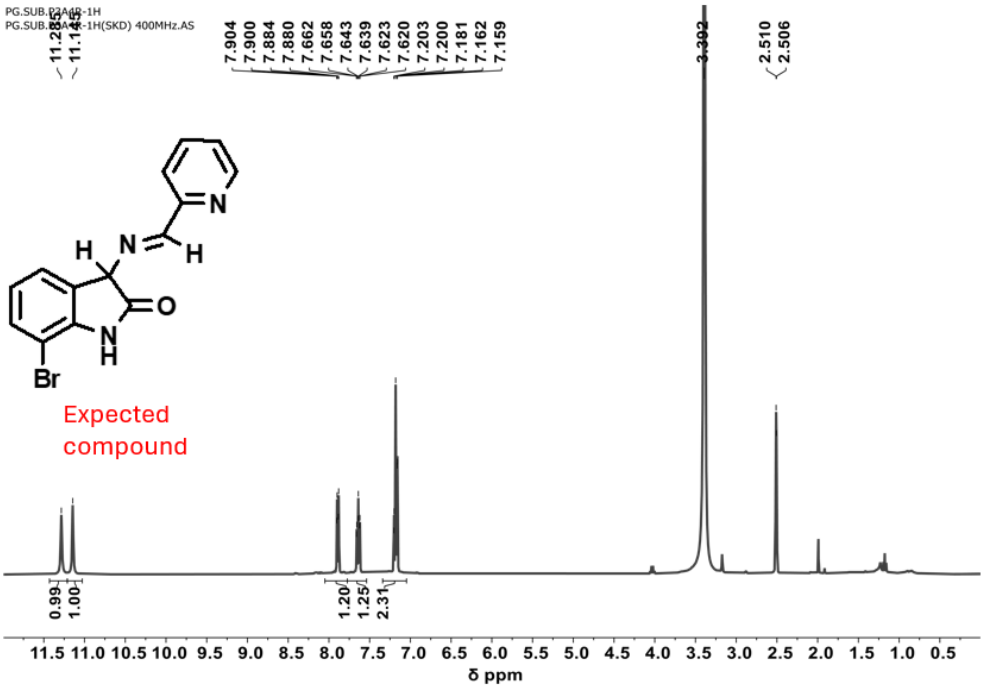


Figure 7: ^{1}H NMR graph of compound 2

The compound here is the expected product but it is not confirmed till now.

According to the NMR graph above, no. of protons present in the original product is much less than the no. of protons present in the expected product.

5.4. Characterization of compound 2 using ^{13}C NMR (150 MHz)

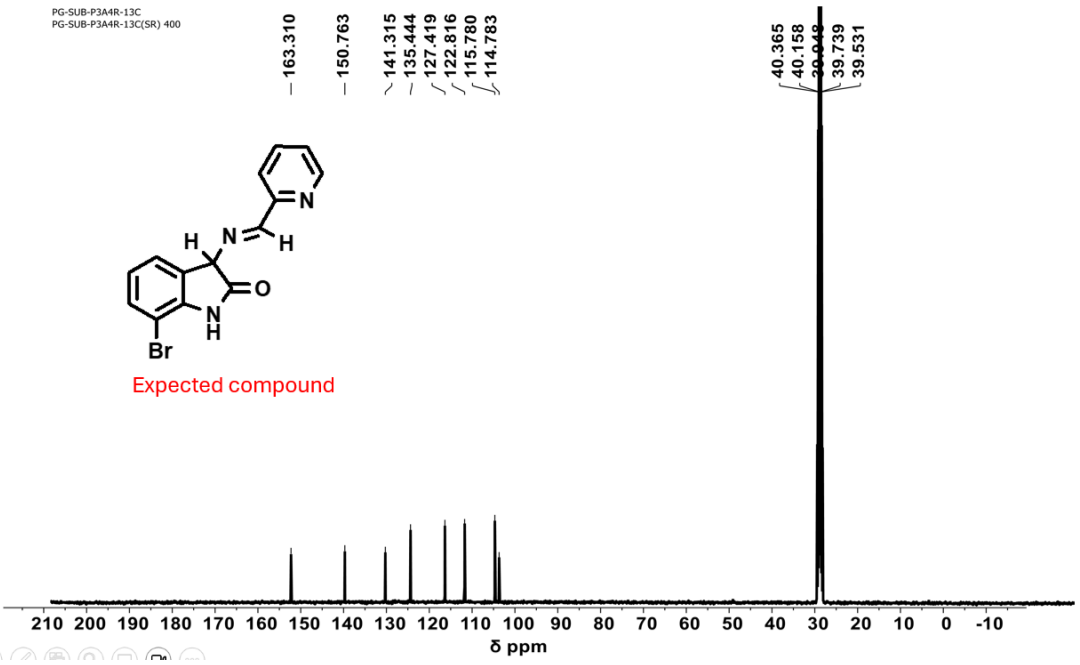


Figure 6: ^{13}C NMR graph of compound 2

Similarly, from the graph above we observed that total no. of Carbon atoms present in the compound is much less than the Carbon atoms present in the expected product.

6. BIOLOGICAL EVALUATION OF COMPOUND

For this, SHSY5Y cells were maintained under standard cell culture conditions. Then the following steps were conducted -

I. Thawing of cells -

For the experiment, the cells were first thawed from cryopreserved stock. Cryovials containing the cells were taken out from liquid nitrogen and agitated at 37°C in water bath till a small crystal floats in the solution. The cell suspensions were transferred to flask or culture dish for seeding. Next day the medium was changed to a fresh medium to avoid the DMSO (present in cryosolution) mediated toxicity to cell.

II. Splitting of cells -

Once the cells have been thawed and are actively growing in culture vessels, they will divide rapidly. So, once the cells reached a confluency of 90-95%, it was ready to split. First they were rinsed with 1X PBS (pH 7.4). Then, 500µl-1ml of 0.25% Trypsin EDTA was added, and the cells were incubated for 5 minutes at 37°C with 5% CO2. Trypsinization was neutralized by adding an equal volume of media. The cells were collected and then pelleted by centrifugation at 1000g for 5 minutes, and the supernatant was discarded. The pellet was resuspended in 1ml DMEM with 10% FBS. For accurate cellular quantification, a 6X dilution was prepared by adding 50µl of trypan blue to 10 µl of the cell suspension. A 10µl aliquot of this diluted sample was then loaded onto a hemocytometer for counting, utilizing a predetermined formula. Finally, cells were seeded at $1x10^5$ cells/ml and cultured in an incubator at 37°C with 5% CO2.

III. Freezing of cells –

Upon reaching a confluency of 85-95%, the cells were dissolved in 10% DMSO in DMEM (supplemented with 10% FBS) in cryovials and stored in liquid nitrogen.

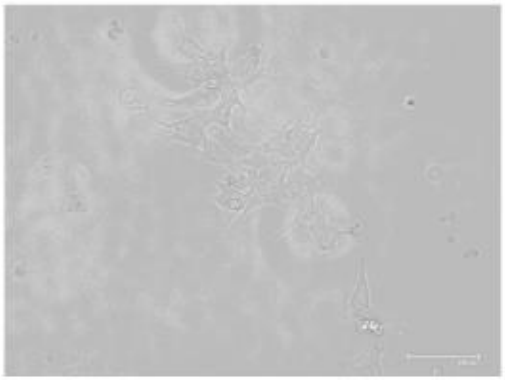
The SHSY5Y cells were then taken from the culture vessels and seeded in 6-well plates at a concentration of 50k cells/well in a 6 well plate and 10-20k cells/well in 96-well plate. After 24 hours, 6 well plates were allocated for the following groups (only DMEM without FBS) –

- Naive Control (media change only)
- Vehicle Control (0.1% DMSO in DMEM),
- 1 µM Compound 1, and
- 100 nM Compound 1.

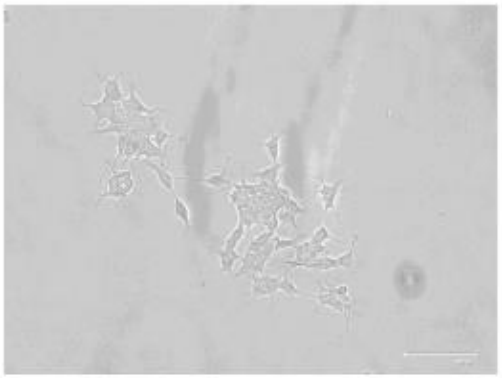
After 48hrs of treatment media was discarded from each plate and 1ml of Trizol reagent was added to each well and collected the cells in designated vials for RNA isolation. Before collection of RNA and adding the trizol, images were captured to analyse the cell morphology to check any alteration upon treatment.

Cell viability analysis (by MTT assay) was conducted, and it was found that our compound is not cytotoxic to the cells.

6.1. Results of biological evaluation

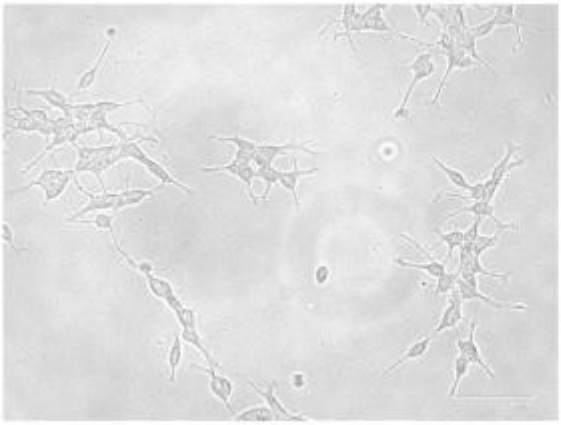


Control

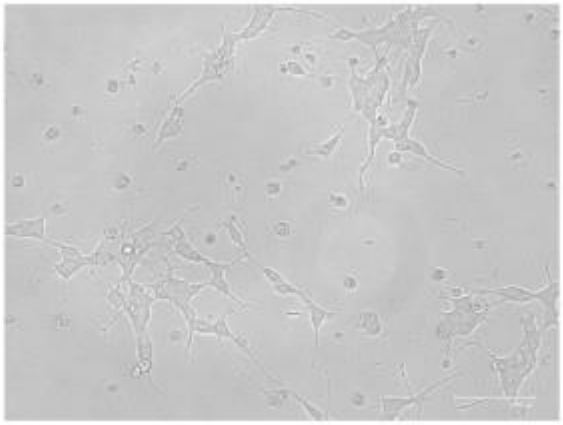


Vehicle control

Figures 9 & 10: Microscopic images of SHSY5Y neuroblastoma cells. Control (Figure 9): after the cells were subjected to media change only – Exhibited normal morphology. Vehicle control (Figure 10): Cells after treating with 0.1% DMSO – Showed no significant morphological alterations as compared to Control.



100 nM



1μM

Figures 11 & 12: Microscopic images of SHSY5Y cells after treating with compound 1 at concentrations of 100nM and 1μM – The cells showed the formation of neuronal networking, with extensive neurite outgrowth, closely resembling the morphology typically induced by serotonin.

We visualized through the images that neuronal networks were forming, and synaptic networks were enhancing.

Since it's an ongoing research, so we need further experiments to confirm if these networks are actually forming.

Conclusion

Serotonin, a vital neurotransmitter, controls plethora of physiological, cognitive, and immune functions. Its dysregulation is intricately linked to numerous severe psychiatric disorders. Despite its extreme importance, a major obstacle in therapeutic intervention lies in serotonin's disability to efficiently cross the blood-brain barrier (BBB). This impermeability severely restricts the direct pharmacological manipulation of central serotonin levels, limiting treatment options for many impairing conditions.

This study directly addresses the challenge of BBB permeability by focusing on the green synthesis of novel neurotransmitter analogues incorporating the isatin moiety. Our approach meticulously prioritized environmentally friendly methodologies, aligning with the imperative for sustainable chemical practices in drug discovery. We designed and synthesized a series of isatin-based compounds with a dual objective: to mimic endogenous serotonin's activity and to efficiently traverse the BBB. The core of our design strategy involved crafting a pharmacophore capable of effectively mimicking serotonin's crucial interactions.

Our synthetic pathway began with the reaction of a bromo-substituted isatin moiety with various amines. These reactions were conducted under green conditions, utilizing water as a solvent and a copper catalyst, reflecting our commitment to minimizing environmental impact.

While purification was diligently performed using column chromatography to maximize product purity, initial Nuclear Magnetic Resonance (NMR) spectroscopy revealed a discrepancy: the number of protons observed in the purified products was consistently lower than theoretically expected for the intended structures while the other compound showed positive results on the NMR graph.

Furthermore, preliminary biological evaluations yielded some promising results, indicating that several of the synthesized isatin-based compounds exhibit significant serotonin mimetic activity and the ability of these novel compounds to effectively cross the BBB.

While further comprehensive in vivo studies are awaited to fully elucidate their pharmacological profiles, efficacy, and safety, the present work strongly supports the potential of the isatin scaffold as a versatile platform for developing next-generation neurotransmitter mimetics with enhanced brain penetrability. This research not only contributes to the field of medicinal chemistry but also underscores the viability of sustainable synthetic strategies in drug discovery.

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ABSTRACT & INTRODUCTION

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Abstract

Neurotransmitters are the body's chemical messengers, transmitting signals across synapses from one neuron to another, profoundly influencing everything from mood and sleep to learning and pain perception. Among these, Serotonin (5-hydroxytryptamine or 5-HT) stands out as a critically important monoamine neurotransmitter. It orchestrates a vast array of physiological, cognitive, and immune functions, with its dysregulation intricately linked to numerous severe psychiatric disorders like depression and anxiety, . However, a formidable barrier in developing effective therapeutics for these Central Nervous System (CNS) related disorders is the blood-brain barrier (BBB). This highly selective semipermeable membrane protects the brain from circulating toxins and pathogens, but simultaneously restricts the passage of many therapeutic agents, including exogenous serotonin, from reaching their targets within the brain. This limitation severely hampers the direct pharmacological manipulation of central serotonin levels, impacting treatment efficacy.

With an eye towards designing pharmacophores capable of mimicking serotonin and efficiently traversing the BBB, this thesis investigates the green synthesis of new neurotransmitter analogues based on the isatin moiety. Our approach meticulously prioritizes environmentally friendly methodologies, embodying the principles of green chemistry for sustainable drug discovery. We propose a synthetic approach to construct new chemical entities where the isatin core acts as a versatile scaffold. This abstract explains the reasoning behind integrating the isatin moiety, known for its diverse biological activities and structural versatility, into novel compounds aimed at imitating serotonin's pharmacological profile. Furthermore, this thesis aims to design molecules in innovative ways, fulfilling the ultimate goal of developing a new generation of CNS-active compounds with improved efficacy and reduced side effects, for promoting advanced therapeutic interventions in neurological and psychiatric conditions. This work not only contributes to medicinal chemistry but also champions eco-conscious pharmaceutical development.

Introduction

The brain's extraordinary range of functions is facilitated by the intercellular communication of neurons, largely accomplished via chemical synaptic transmission [1]. This is done by neurotransmitters which are the body's chemical messengers. Neurotransmitters play a crucial role in the functioning of the nervous system which oversees the control and coordination of all bodily activities [2]. A traveling action potential at the axon terminal, induces the synaptic release of neurotransmitters [2]. Eventually the neurotransmitter diffuses across the synapse and binds to receptors on the postsynaptic neuron. This interaction can either excite or inhibit the activity of the postsynaptic neuron [2].

Neurotransmitters are fundamental for control of behavioural and physiological functions in the body

Page 1

that are essential for survival [3]. The functions of neurotransmitters are directly linked to the central nervous system that controls the human body and directly supervise neurophysiological processes such as sleep, emotions, memory, and other cognitive functions [4,5]. They play essential roles in the functioning of the brain, being fundamental regulators of neuronal growth, differentiation, and survival. Consequently, abnormal levels of NTs are reflected in dysregulation of brain functions, leading to various physical, psychotic, and neurodegenerative diseases [6]. Some CNS-related disorders such as epilepsy, multiple sclerosis, Alzheimer's and Parkinson's disease can occur due to NT imbalances.

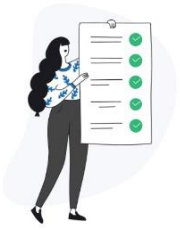
Serotonin, or 5-hydroxytryptamine is one such important monoamine neurotransmitter which plays a crucial role in various physiological functions such as regulating mood, sleep patterns, sexuality, anxiety, appetite and pain. Serotonin is a key messenger in the brain that is associated with behaviour and cognitive functions [7]. Serotonin, in conjunction to other neurotransmitters, like dopamine, affects sleep quality and also modulates glutamate and GABA transmission. Serotonin affects a wide variety of immune functions; it can affect systemic inflammation, as well as a number of autoimmune diseases [9]. Serotonin regulates behavior by modulating neuronal excitability and plasticity, and its dysfunction has been implicated in several psychiatric disorders, such as impulsive aggression, anxiety and depression [10]. Serotonin modulation can, therefore, be potentially useful as a therapeutic strategy [9].

Problems associated with neurotransmitters

There are many problems associated with neurotransmitters. A significant challenge is serotonin's inability to cross the blood-brain barrier [9].

The blood–brain barrier (BBB), made up of endothelial cells, is a dynamic boundary between the brain and the surrounding tissues which plays a critical role in maintaining normal physiological functions of the brain. Endothelial cells strictly regulate the movement of substances into and out of the brain through tight junctions, maintaining the selective permeability of the blood–brain barrier [11]. It safeguards the CNS against circulating toxins, pathogens, and xenobiotics [12]. Additionally, it supplies the brain with nutrients and expels waste products back into the bloodstream [12]. The BBB establishes a very delicate ion homeostasis and adjusts the level of neurotransmitters, which guarantee ideal function of the CNS [12]. It also prevents many therapeutic agents from reaching their targets in the brain that have been developed and could be used to treat various neurological disorders [12,13]. The blood–brain barrier greatly restricts influx of some neurotransmitters such as serotonin but allows the influx of many others [14].

This research focuses on creating new Pharmacophore designed to replicate the function of serotonin, with the aim of treating neurodegenerative disorders like depression, Parkinson's disease, and Alzheimer's disease. It aims to develop a Pharmacophore that could more effectively mimic serotonin's effects and be easily capable to cross the BBB. The primary compounds utilized in this reaction was isatin and amines as starting materials.



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Page 2

AIMS AND OBJECTIVE, DESIGNING ASPECT, MATERIALS AND METHOD

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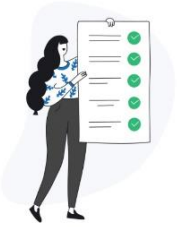
AIMS AND OBJECTIVE
1) Design and Synthesis of the compound: One important aspect of my research involves designing and the synthesis of the compound using green chemistry, focusing on environmental stability.
• This process reduces chemical waste and minimizes the need for extensive purification steps.
• Copper was used as catalyst, which was recovered and reused, promoting resource conservation.
2) Characterization of the compound:
• Nuclear Magnetic Resonance (NMR) and mass spectroscopy was used to determine the molecular structure of the synthesized compound.
• The new pharmacophores were fully purified and characterized before proceeding to biological tests.
3) Biological evaluation of the compound:
• The synthesized compound represents a probable advanced therapeutic agent.
• Ongoing biological testing aims to evaluate the pharmacophores' therapeutic potential, particularly their ability to mimic serotonin in natural cells.
The 3 neurotransmitters shown in Fig 1, Tryptophan is the precursor of serotonin and melatonin. All of them share some common functional groups which are the indole ring and amine (-NH2). But among these 3, only serotonin cannot cross the blood brain barrier while melatonin and tryptophan can. So we try to design a model where we can introduce indole ring and amine group.
We have chosen bromo-substituted isatin moiety which has one indole ring and two active centers for amine incorporation. One is the bromide center, and another one is ketone center. So, we planned to react this molecule with amines such as 2 Picolylamine and N,N-Dimethylaniline. The interaction of these functional groups is expected to form a stable product that further modifies the original indole structure, enhancing the potential to cross the blood-brain barrier.
2.2.1. Procedure
452.06 mg 7-bromo isatin, 420 uL 2 picolylamine, approx... 5 ml distilled water and catalytic amount of copper powder were mixed in a 25 ml round bottom flask. Next the flask was fitted in a reflux condenser to prevent solvent loss and maintain a controlled reaction environment. The reaction was carried out in a magnetic stirrer with an oil bath for 18 hours and temperature is gradually raised and maintained at approx..120°C. In this reaction, copper served as a catalyst to facilitate the reaction between 7-Bromoisatin and 2 picolylamine, with water acting as the solvent. Using water as a solvent fulfils green chemistry principles, as it reduces dependence on harmful organic solvents that can cause harm to environment.
2.2.2. Reaction process
The progress of the reaction was monitered at some intervals by TLC (thin layer chromatography) method. In this process, a small sample of the reaction mixture was taken and spotted onto a TLC plate alongside the starting material and product. The Plate was then developed in an appropriate solvent system made of hexane and ethyl acetate. The components on the plate move upwards by capillary action. The new spots/bands which were formed on the TLC plate, were then observed under the UV light. The disappearance of the starting material's spot and appearance of new spot corresponding to the desired product indicate the progress of the reaction.
2.2.3. Work up
In this process, initially the water was evaporated using rotary evaporator to get the crude product. The catalyst was then recovered by isolation of crude product using organic solvents such as mixture of ethyl acetate, methanol and chloroform. The organic layer is decanted off for preparation of slurry for column chromatography. The Cu powder remains intact in this mixture.
2.2.4. Purification
Column chromatography technique was used to isolate the nanodrug and eliminate impurities. This technique separates compounds based on their separation of stationary phase (silica) and mobile phase (ethyl acetate and hexane). This approach was particularly effective in distinguishing the desired product from any unreacted starting material or byproducts. TLC was utilized throughout the process to track the reaction's progress and desired compound was being produced.
For the purification of the compound, a clean chromatography column was prepared by washing with pure hexane and then drying. A small plug of cotton was then positioned at the bottom of the column to support the stationary phase. A slurry of silica gel, prepared in hexane, was carefully loaded into the column. Another cotton plug was put on top of the silica bed leaving some gap above the silica bed. The column was then eluted with a gradient of hexane and ethyl acetate. The solvent polarity was gradually increased based on thin-layer chromatography (TLC) results to achieve optimal separation. The purified nanodrug was subsequently collected and prepared for further characterization.



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BIOLOGICAL EVALUATION OF COMPOUND

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I. Thawing of cells -
For the experiment, the cells were first thawed from cryopreserved stock. Cryovials containing the cells were taken out from liquid nitrogen and agitated at 37°C in water bath till a small crystal floats in the solution. The cell suspensions were transferred to flask or culture dish for seeding. Next day the medium was changed to a fresh medium to avoid the DMSO (present in cryosolution) mediated toxicity to cell.
II. Splitting of cells -
Once the cells have been thawed and are actively growing in culture vessels, they will divide rapidly. So, once the cells reached a confluency of 90-95%, it was ready to split. First they were rinsed with 1X PBS (pH 7.4). Then, 500µl-1ml of 0.25% Trypsin EDTA was added, and the cells were incubated for 5 minutes at 37°C with 5% CO2. Trypsinization was neutralized by adding an equal volume of media. The cells were collected and then pelleted by centrifugation at 1000g for 5 minutes, and the supernatant was discarded. The pellet was resuspended in 1ml DMEM with 10% FBS. For accurate cellular quantification, a 6X dilution was prepared by adding 50µl of trypan blue to 10 µl of the cell suspension. A 10µl aliquot of this diluted sample was then loaded onto a hemocytometer for counting, utilizing a predetermined formula. Finally, cells were seeded at $1x10^8$ cells/ml and cultured in an incubator at 37°C with 5% CO2.
III. Freezing of cells –
Upon reaching a confluency of 85-95%, the cells were dissolved in 10% DMSO in DMEM (supplemented with 10% FBS) in cryovials and stored in liquid nitrogen.
The SHSY5Y cells were then taken from the culture vessels and seeded in 6-well plates at a concentration of 50k cells/well in a 6 well plate and 10-20k cells/well in 96-well plate. After 24 hours, 6 well plates were allocated for the following groups naive control (only media change without FBS) –
- Naive Control (media change only)
- Vehicle Control (0.1% DMSO in DMEM),
- 1 µM 5HT,
- 10 µM HT,
- 1 µM Compound 2, and
- 10 µM Compound 2.

After 48hrs of treatment media was discarded from each plate and 1ml of Trizol reagent was added to each well and collected the cells in designated vials for RNA isolation. Before collection of RNA and adding the trizol, images were captured to analyse the cell morphology to check any alteration upon treatment.
Cell viability analysis (by MTT assay) was conducted and it was found that our compound is not cytotoxic to the cells.

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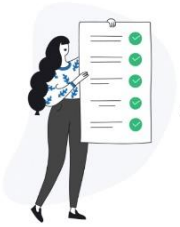
We visualized through the images that neuronal networks were forming, and synaptic networks were enhancing.
Since it's an ongoing research, so we need further experiments to confirm if these networks are actually forming.



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RESULTS AND DISCUSSION AND CONCLUSION

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A small sample of the dried product was set aside to be analysed by using NMR spectroscopy. To obtain accurate and interpretable spectra, the sample was dissolved in an appropriate solvent (like DMSO-d_6), moved to a clean NMR tube, and prepared according to standard procedures

5.1. Characterization of compound 1 using 1H NMR (600 MHz)

The graph above, indicates that –

 On the graph, b at δ 2.91 ppm represents the aliphatic protons that is the H from N-CH3 groups. Total 6H from each group.

 The peaks present between δ 6.63 and δ 7.40 ppm represents the 11 aromatic protons that is the H present in the aromatic rings.

 The peak a at δ 10.68 ppm represents an acidic proton, 1H associated with -NH.

5.2. Characterization of compound 1 using 13C NMR (150 MHz)

Figure 6: 13C NMR graph of compound 1

The graph above, indicates that –

 On the graph, b at δ 62.11 ppm corresponds to a methyl group attached to a Nitrogen atom (N-CH3).

 The peak c at δ 102.53 ppm indicates a quarternary carbon.

 The peaks marked as 'Aromatic carbons' between δ 112.62 ppm and δ 149.82 ppm represents the Carbon atoms in the aromatic rings.

 The peak a at δ 179.57 ppm represents a carbonyl carbon (C=O).

5.3. Characterization of compound 2 using 1H NMR (600 MHz)

Figure 7: 1H NMR graph of compound 2

The compound here is the expected product but it is not confirmed till now.

According to the NMR graph above, no. of protons present in the original product is much less than the no. of protons present in the expected product.

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Figure 6: 13C NMR graph of compound 2

Similarly, from the graph above we observed that total no. of Carbon atoms present in the compound is much less than the Carbon atoms present in the expected product.

Conclusion

Page 1 ‹

Serotonin, a vital neurotransmitter, controls plethora of physiological, cognitive, and immune functions. Its dysregulation is intricately linked to numerous severe psychiatric disorders. Despite its extreme importance, a major obstacle in therapeutic intervention lies in serotonin's disability to efficiently cross the blood-brain barrier (BBB). This impermeability severely restricts the direct pharmacological manipulation of central serotonin levels, limiting treatment options for many impairing conditions.

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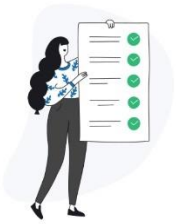

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